Close contact fluctuations: the seeding of signalling domains in the immunological synapse

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We analyse the size and density of thermally induced regions of close contact in cell:cell contact interfaces within a harmonic potential approximation, estimating these regions to be below 1/10 th of a micron across. Our calculations indicate that as the distance between the close contact threshold depth and the mean membrane-membrane separation increases, the density of close contact patches decreases exponentially while there is only a minimal variation in their mean size. The technique developed can be used to calculate the probability of first crossing in reflection symmetry violating systems.

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Surface contact between cells is a key mechanism for information transfer in many biological systems. These can be both long term or permanent connections as in the neurological synapse, or as discovered more recently, transient and highly dynamic as in the immunological synapse [1]. T-cells (a class of lymphocytes) make transient contact with 'target' cells whilst scanning for the presence of their specific antigen, antigen recognition resulting in the stabilisation of the contact and generation of a macroscopic receptor patternation in the contact interface, or a so called immunological synapse [1]. A fundamental observation is that the contact interface is heterogeneous, both in the physical separation of the two cell surfaces [2] and in the local signalling properties [1, 3, 4]. Differences in the extracellular lengths of key molecules/bonds is believed to underpin both these processes with a predominant division between short and long bond length molecular species. Of note is that essential antigen signalling receptors, such as the T-cell receptor (TCR), are small molecules with a ligand-receptor bond length of 14nm (membrane to membrane span) [1], while an essential phosphatase (CD45), a major component of the glycocalyx, has a length of 25-40nm and is not believed to have a natural ligand. T cell signalling, or antigen detection, thus requires tight cell:cell contact to allow TCR binding, whilst such regions necessarily require the spatial exclusion of the large molecules comprising the glycocalyx. Spatial heterogeneity in the membrane profile within the contact interface is therefore essential for the functioning of the cell contact. Early patterns (50sec) in cell interfaces show random small clusters of TCRs [3, 4], regions where signalling intermediaries appear to congregate. These regions of close contact are presumably formed from fluctuations in the initial contact surfaces. At later times signalling appears to be focused in distinct stable microclusters [5]. This dependence of signalling on spatial heterogeneity introduces a key 'exposure' problem; ligand detection requires that regions of close contact comprise a significant area within the interface while they must be sufficiently large that they can be stabilised when segregation is energetically favourable [6]. We examine the spatial statistics of these regions of close contact using a linear stochastic model for thermal fluctuations of the membrane separation.

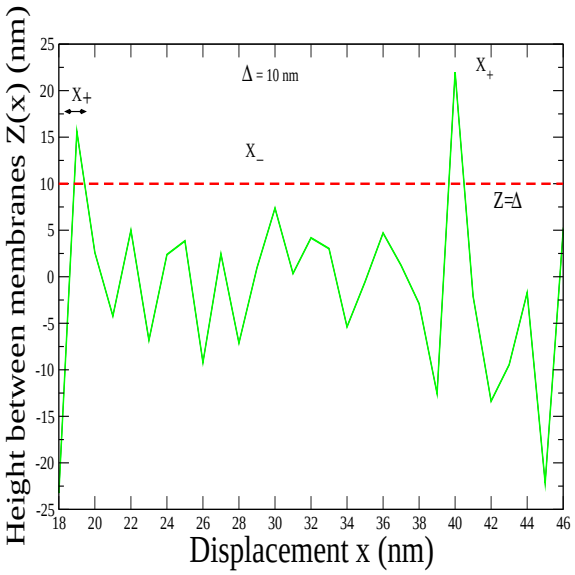

FIG. 1: A 1D profile of a 2D membrane fluctuating around the line $< Z >= 0$, from simulated data. X_+ & X_- are the respective sizes of the patches above and below the threshold depth $Z = \Delta = 10$nm.

In this letter, our interest is in the size and density of regions of close contact (eg membrane-membrane separation <20 nm) where effective TCR ligand binding can occur. We utilise a linearised version of the synapse reaction-diffusion equations [6, 7] to model pre pattern dynamics, reducing to a single equation for the membrane-membrane separation Z around a mean separation (25-50nm) determined by the glycocalyx potential and receptor-ligand bond equilibrium. In this regime, the fluctuation $Z(\vec{x}, t)$ has dynamics

$$M\frac{\partial Z}{\partial t} = -B\nabla^4 Z + \tau\nabla^2 Z - \lambda Z + \eta \qquad (1)$$

where B is the membrane rigidity, τ the surface tension, M the membrane damping constant and λ parametrises the rate of relaxation of the membranes close to equilibrium, *i.e.* the strength of the harmonic approximation to the potential well. The thermal noise $\eta(\vec{x},t)$ is defined using a fluctuation-dissipation relation $< \eta(\vec{x},t)\eta(\vec{x}',t') >= 2k_BTM\delta^2(\vec{x}-\vec{x}')\delta(t-t')$, $\vec{x},\vec{x}'$ being points in the contact interface. The solution $Z(\vec{x},t)$ is a Gaussian variate. We wish to calculate the probability that the displacement $Z(\vec{x},t)$ lies below a 'close contact' threshold $-\Delta$ where $\Delta \sim 5-30$ nm is the membrane-membrane displacement from the mean required for efficient TCR binding. We identify the region $Z < -\Delta$ as a region of close contact and determine the average size of these close contact patches. A point to note is the symmetry violation of the system around $Z = -\Delta$; specifically the average size of a patch above this line (designated by $+$) is not the same as one below this line (designated by $-$). The statistics for $Z < -\Delta$ are identical to those for $Z > \Delta$; thus for presentation we will use $Z > \Delta$ as the threshold.

We start by defining the sign (conditional) correlator for an arbitrary displacement $\vec{x}$ in the contact interface (relative to the origin) [8, 9] $A_+ =< \mathrm{sgn}[Z(\vec{x}) - \Delta] >_{Z(\vec{0})>\Delta}$ and $A_- =< \mathrm{sgn}[Z(\vec{x})-\Delta] >_{Z(\vec{0})<\Delta}$, $< .. >_F$ denoting the average over states where condition F holds. For simplicity we assume Z is in stationary equilibrium and thus initial conditions can be ignored. $Z(\vec{0})$, $Z(\vec{x})$ define a two variable joint Gaussian probability distribution with zero means, variance $c_{11} =< Z^2(0) >$ and covariance $c_{12}(\vec{x}) =< Z(0)Z(\vec{x}) >$. By translational symmetry the covariance matrix and $A_\pm$ only depend on the spatial displacement $x = |\vec{x}|$ between the membranes. Thus we drop explicit reference to $\vec{x}$ for simplicity. The symmetry relation $A_+(x,\Delta) = -A_-(x,-\Delta)$ means that only A_+ needs to be evaluated.

An ensemble averaging over the two-variable Gaussian distribution gives

$$\begin{aligned} A_+(x) &= \frac{N_+}{\sqrt{2\pi c_{11}}}\int_{-\infty}^{\infty} du\ sgn(u-\Delta)\exp(-\frac{u^2}{2c_{11}}) \\ &\times \int_{(\Delta-u\frac{c_{12}(x)}{c_{11}})(\frac{c_{11}}{\det c})^{1/2}}^{\infty} dz\frac{\exp(-z^2/2)}{\sqrt{2\pi}} \end{aligned} \quad (2)$$

where the lower limit follows from the condition $Z(\vec{0}) > \Delta$. Here $\det c = c_{11}^2 - c_{12}^2$ and the normalisation constant N_+ is defined by the error function ${N_+}^{-1} = \int_{\frac{\Delta}{\sqrt{c_{11}}}}^{\infty} du\frac{\exp(-u^2/2)}{\sqrt{2\pi}}$ which is in fact the probability of observing a separation $Z(\vec{x}) > \Delta$ at an arbitrary point $\vec{x}$. We define the patch sizes $X_\pm$ for regions where $Z > \Delta, Z < \Delta$ respectively (in 2D along an arbitrary vector), Fig. 1. To evaluate $< X_\pm >$, we need to evaluate $A'_\pm(0,\Delta)$ where the prime refers to a derivative with respect to $x = |\vec{x}|$. This follows from the relation $A_\pm(x,\Delta) = 1 - 2x/ < X_\pm >$ as separation $x \to 0$, a consequence of the fact that the probability of finding a crossing (*i.e.* $Z = \Delta$) in a small interval of length x is $\frac{x}{<X_\pm>}$. This gives us the exact relation $< X_\pm >= -\frac{2}{A'_\pm(0)}$.

We proceed to compute the derivative as $A'_\pm(x) = \frac{\partial A_\pm}{\partial c_{12}}.\frac{\partial c_{12}}{\partial x}$, Eq. (2) giving

$$\frac{\partial A_+}{\partial c_{12}} = \frac{N_+}{\pi}\ \frac{\exp[-\frac{\Delta^2}{2c_{11}}]}{\sqrt{\det\ c}}\ \exp[-\frac{c_{11}}{2\det\ c}\Delta^2(1-\frac{c_{12}}{c_{11}})^2] \quad (3)$$

The relevant correlator in 2D is given by

$$\begin{aligned} c_{12}(x) &= \frac{k_BT}{(2\pi)^2M}\int d\vec{k}\frac{e^{-i\vec{k}\cdot\vec{x}}}{\alpha(\vec{k})} \\ &= \frac{k_BT}{4\pi\sqrt{\lambda B}}\frac{K_0(e^{-\phi/2}\hat{x}) - K_0(e^{\phi/2}\hat{x})}{\sinh\phi} \end{aligned} \quad (4)$$

where $\alpha(\vec{k}) = \frac{B|\vec{k}|^4+\tau|\vec{k}|^2+\lambda}{M}$, $\phi = \log\left(\frac{\tau-\sqrt{\tau^2-4\lambda B}}{2\sqrt{\lambda B}}\right)$, $\hat{x} = \left(\frac{\lambda}{B}\right)^{\frac{1}{4}}x$ and K_0 is a modified Bessel function of degree 0. The final integral uses a Bessel function identity [10]. Therefore $c_{11} = \frac{k_BT\phi}{4\pi\sqrt{\lambda B}\sinh\phi}$, and for small x we find $A_+(x,\Delta) \sim 1 - \hat{x}(\log_e\hat{x})^{\frac{1}{2}}C$, C a constant. Thus crossings fail to conform to the assumptions above, specifically c_{12} is not twice differentiable at $x = 0$ [11]. This is a familiar consequence of Brownian motion crossing behaviour and stems from the high frequency noise component of η that causes repeated crossing of the threshold in between large excursions away from the threshold. We regularise the divergence by introducing an infra-red cut-off in the noise, thus correlator (4) becomes

$$c_{12}(x) = \frac{k_BT}{2\pi M}\int_0^{k_m} dk\frac{kJ_0(xk)}{\alpha(k)}, \quad (5)$$

where J_0 is a Bessel function of degree 0 and cut-off $k_m = 2\pi/\epsilon$ is given by the smallest length scale ϵ in the system. This length scale is on a sub nanometer scale, e.g. the width of lipid molecule head in the membrane. A regular expansion for c_{12} at small x now follows

$$\begin{aligned} &c_{12} \sim c_{11} - \\ &x^2\left(\frac{k_BT}{32\pi B}\log_e\left(\frac{Bk_m^4+\tau k_m^2+\lambda}{\lambda}\right) - \frac{\tau}{8B}c_{11}\right) \end{aligned} \quad (6)$$

Provided ϵ is sufficiently small we have a consistent regularisation with $c''_{12} < 0$ at $x = 0$. We thus obtain the following expression

$$A'_+(0,\Delta) = -2\frac{N_+}{f}\ (\frac{\lambda}{B})^{1/4}\ \exp(-\Delta^2/2c_{11}) \quad (7)$$

where $f = 4\pi\sqrt{\phi/\left(log\left(\frac{k_m^4B}{\lambda}\right)\sinh\phi\right)}$ depends only on system parameters. We have retained only the leading

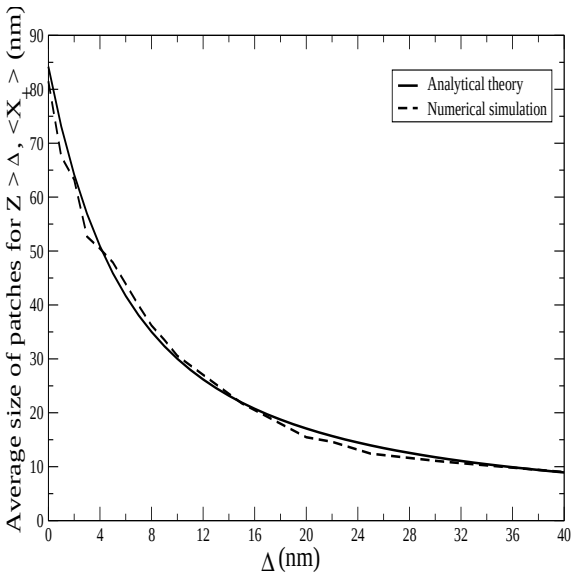

FIG. 2: Variation of $< X_+ >$ against Δ for $Z > \Delta$: theoretical estimate from Eq. (8) vs numerical simulation taken on a lattice, size 1000, spacing 1nm, over 1024 runs. A coloured noise spectrum is used, derived by projection from 2D, giving $< \eta(x,t)\eta(x',t') >= 2k_BTMs(x-x')\delta^2(\vec{x}-\vec{x}')\delta(t-t')$ with $s(k) = (\frac{M\alpha(k)}{B})^{1/4} \frac{1}{4\cosh\frac{1}{2}\hat{\phi}(k)}$, where $\hat{\phi}(k) = \log[\frac{\tau+2Bk^2}{\sqrt{4BM\alpha(k)}} - \sqrt{\frac{(\tau+2Bk^2)^2}{4BM\alpha(k)} - 1}]$.

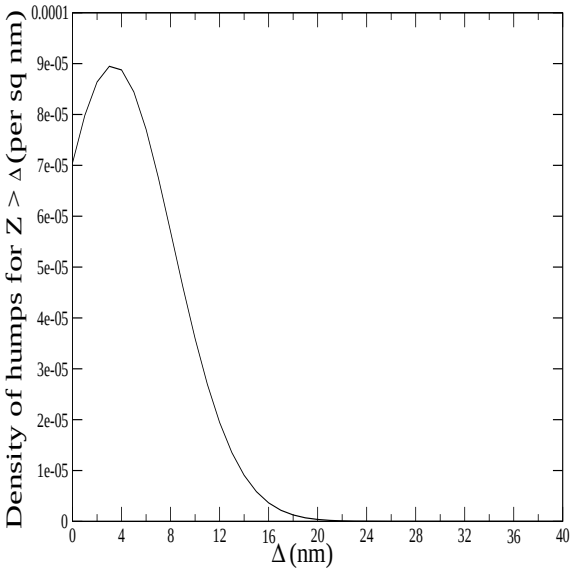

FIG. 3: Probability density of humps around the line $Z = \Delta$ as a function of the average threshold Δ (nm) as predicted from Eq. (9)

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order in the cut-off for simplicity. The mean sizes of the patches above and below the line $Z = \Delta$ now follow,

$$< X_\pm >= \frac{f}{N_\pm}(\frac{B}{\lambda})^{1/4} \exp(\Delta^2/2c_{11}) \qquad (8)$$

where the normalisation constant N_- is defined as ${N_-}^{-1} = 1 - {N_+}^{-1}$. The dependence on the cut-off is weak while the length scale is determined by $\sqrt{c_{11}B/k_BT}$. For a symmetry preserving system with $\Delta = 0$ we have $< X_\pm >= \frac{f}{2}(\frac{B}{\lambda})^{1/4}$. Suitable values for the system parameters are [6] : $B = 11.8\ k_BT$, $\tau = 5650 k_BT\ \mu m^{-2}$, $M = 4.7 \times 10^6\ k_BT\ s\ \mu m^{-4}$ and $\epsilon = 1$nm, while $\lambda = 6.0 \times 10^5\ k_BT\ \mu m^{-4}$ is approximated from the linearised reaction-diffusion equation as $\tau\times$ CD45 density, the latter being approximately 100 molecules μm^{-2}. This follows from the force expression in synapse reaction diffusion equations, $\sum_i \kappa(z - l_i)C_i$, a sum over all molecules C_i that impose a force on the membrane (bond length l_i) with a spring constant $\kappa \sim \tau$, [6, 12]. In early signalling, CD45 will be the dominant component. These values give $f = 2.5$, $< X_\pm(\Delta) > |_{\Delta=0} \sim 84$ nm. The variation of $< X_\pm(\Delta) >$ with Δ is illustrated in Fig. 2. Density fluctuations in the C_i will causes fluctuations in λ which can be included as a "non-equilibrium temperature" in Eqn. (1) (fluctuation-dissipation relation); however this is beyond the current minimalist model.

As the threshold Δ increases above zero the regions $Z > \Delta$ develop into isolated patches in 2D. We can use the mean size to estimate the patch density $\rho_{\rm humps}$ by a mean field approximation $\rho_{\rm humps} < X_+ >^2 = {N_+}^{-1}$ to obtain

$$\rho_{\rm humps}{}^+ = \frac{N_+}{f^2}(\frac{\lambda}{B})^{1/2} \exp(-\frac{\Delta^2}{c_{11}}). \qquad (9)$$

The expected decline in the density of patches as Δ increases is shown in Fig. 3. For large $\Delta \gg c_{11}$ the leading behaviour is $< X_+(\Delta) > \sim f\frac{c_{11}^{\frac{1}{2}}}{\sqrt{2\pi}\Delta}(\frac{B}{\lambda})^{1/4}$ and $\rho_{\rm humps} \sim \frac{\Delta}{f^2}\left(\frac{2\pi\lambda}{c_{11}B}\right)^{1/2} \exp(-\frac{\Delta^2}{2c_{11}})$. These asymptotic approximations capture the contrasting weak decline of the width $< X_+ >$, and strong decay of the hump density $\rho_{\rm humps}$ with Δ in Figs. 2 & 3. In 1D, regions with $Z > \Delta$ are always disconnected so the patch density $\rho_{\rm humps}$ can be defined for all values of Δ. Further, in 1D, there are no divergences, whilst in higher dimension the divergences are more severe. These properties result from the interplay between the 4^{th} order PDE Eq. 1 and the volume of phase space.

The probability density function of the distance between crossings can also be approximated. This utilises the probability distribution for the number of crossings of the line $Z = \Delta$, which is computed by generalising the traditional 'persistence' analysis [8, 9]. We need to discriminate between the two types of crossings, a crossing from $Z > \Delta$ to $Z < \Delta$ as x increases, and the converse. Let $p_n^+(x)$ denote the probability that an interval of length x contains n crossings of $Z(\vec{x}, t)$ across the reference level Δ when $Z > \Delta$ at the extreme left, and $p_n^-(x)$

is the corresponding probability with $Z < \Delta$ at the extreme left; in 2D we consider moving a distance x along a specified vector. Then under an independent interval approximation, for $n \geq 1$, their Laplace transforms have the forms

$$\begin{aligned} \tilde{p}_n^{\pm}(s) &= \frac{N_{\pm}}{2} \frac{(1-\tilde{P}_+)(1-\tilde{P}_-)\left(\tilde{P}_+ \ \tilde{P}_-\right)^{\frac{n-1}{2}}}{s^2 < X >}, \\ &= \frac{N_{\pm}}{2} \frac{(1-\tilde{P}_{\pm})^2 \ \tilde{P}_{\mp}\left(\tilde{P}_+\tilde{P}_-\right)^{\frac{n-2}{2}}}{s^2 < X >}, \end{aligned} \tag{10}$$

for n odd and even respectively. Here $P_{\pm}(x)$ is the probability density for the distance x between crossings ($+ : Z > \Delta$, $- : Z < \Delta$), and $< X >= (< X_+ > + < X_- >)/2$ is the average distance between consecutive crossings (any type). Using the identities $\sum_{n=0}^{\infty} \ p_n^{\pm}(X) = 1$, we can now show that $\tilde{p}_0^{\pm} = s^{-1} - N_{\pm}(1-\tilde{P}_{\pm})/(2s^2 < X >)$, which agrees with [9] when $\Delta = 0$. Employing the identity $A_{\pm}(x) = \sum_{n=0}^{\infty} \ (-1)^n \ p_{\pm}(x)$, we arrive at two coupled equations relating $P_{\pm}$ and $A_{\pm}$,

$$\tilde{A}_{\pm}(s) = \frac{1}{s} - \frac{N_{\pm}}{s^2 < X >} \frac{(1-\tilde{P}_-)(1-\tilde{P}_+)}{1-\tilde{P}_+\tilde{P}_-} \tag{11}$$

Solving these equations then gives the desired pdfs $P_{\pm}(x)$.

To summmarize, for the harmonic potential membrane model we have an exact analytic calculation for the mean size of close contact patches, $< X_+(\Delta) >$, our calculations suggesting that these are on the scale of tens of nm. The scale is primarily determined by the combination $\sqrt{c_{11}B/k_BT}$ and has a leading order behaviour going as $1/\Delta$ for large Δ. This small patch size implies that multiple receptor bindings are unlikely within a patch and close contact patches are unobservable by traditional light microscopy. The small size also implies that phosphatase exclusion (CD45) probably results from density fluctuations, ie a specific exclusion mechanism is not required in contrast to that needed at larger sizes [6, 7]. The density of patches decays rapidly with the threshold Δ on a length scale of $\sqrt{c_{11}} \sim 5.4nm$, Eq. (8), and indicates that cell membranes must be highly flexible otherwise the glycocalyx would impose too large a barrier to allow formation of close contact regions (λ increasing with membrane elasticity). In particular, the glycocalyx cannot be too deep relative to the size of the TCR ligand-receptor bond length (14nm) since otherwise the density of patches becomes too small for antigen detection. The probability of T cell signalling depends on the ability of the TCR to bind it's ligand and is thus crucially dependent on the area of close contact regions within the cell:cell interface which varies as N_+^{-1}, and on the size of those close contact patches. There is an enhancement in triggering as patch sizes increase above 150nm [13]; thus our estimates suggest that early signalling relies on patches below this size and enhancement effects only occur upon aggregation and stabilisation of clusters as the immunological synapse forms. Such conclusions are somewhat reminiscent of [12] where formation of a synapse was related to a critical value of the system parameters (albeit without evaluating the patch size). We, however, go beyond such qualitative predictions. Our calculations clearly suggest that the membrane correlation length is a determining factor in the area of close contact regions in the interface, which with our parameters limits the threshold to $\Delta < 16$ nm.

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